# Chapter 5.3

# Ambient toxicity of sediments from the Maryland Coastal Bays

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## Abstract

Overall, the Coastal Bays sediments show little evidence of toxicity. This is consistent with the sediment chemistry results that there were no exceedances of ER-M values. It is important to note that dead-end canals were not sampled; other studies have shown these areas to have more toxicity due to leachate from pilings and runoff from development (Challiou et. al. 1996).

## Introduction

Ambient toxicity is a bioassay that is used to evaluate potential toxicity by exposing an indicator organism to surficial sediment samples and measuring mortality and/or growth over time. The survival rate of the amphipod *Ampelisca abdita* is measured in the sediments in a 10-day assay and compared to a control sample, which uses sediment from a relatively clean reference site. Samples differing significantly from the control were considered to have significant toxicity.

There had never been any ambient toxicity study done on the Maryland Coastal Bays before 1999. During the summer of 1999, DNR conducted a pilot study comparing two sediment toxicity bioassays from five stations in the Maryland's Coastal Bays (Figure 5.3.1). Comparison was made with sediment from a control site, Fishing Bay with Patuxent River sand (25%). Two different amphipod species were used; *Leptocheirus plumulosus* and *Ampelisca abdita* to determine which organism was a better indicator in the coastal bays (*L. plumulosus* is used in Chesapeake Bay monitoring and *A abdita* is used for EMAP monitoring).

*Ampelisca abdita* is a tube-dwelling amphipod found mainly in protected areas from the low intertidal zone depths to 60m. It ranges from central Maine to south-central Florida and the Eastern Gulf of Mexico and has also been introduced into San Francisco Bay. It has been reported in waters, which range from fully marine to 10 parts per thousand salinities and inhabits sediments from fine sand to mud and silt without shell, although it may also be found in relatively coarser sediments with a sizable fine component. *A. abdita* may be collected throughout the year. For these reasons, this is the methodology used by USEPA for national assessments.

The 10-day survival and growth *Leptocheirus plumulosus* test (and subsequently 28-day survival, growth and reproduction test) was also used to assess the toxicity of the coastal bays stations because this is the technique used in Maryland and the Chesapeake Bay. *Leptocheirus plumulosus* is an estuarine amphipod found in fine-grained clayey-silt sediments with moderate organic carbon content along the East coast. It also inhabits areas with wide range of salinities (0-33ppt). Although *Ampelisca* has a similar salinity range, it slightly differs from *Leptocheirus* in temperature range. *Ampelisca* has slightly colder test temperature requirement.

Since that study was never published, the results are presented in this document.

**Data Sets**

Environmental Monitoring and Assessment Program, EMAP

EMAP: Joint Assessment of the Maryland and Delaware Coastal Bays 1996

EMAP: Mid-Atlantic Integrated Assessment, MAIA 1997-98

July 1999 Preliminary DNR study – tested new methods in preparation for August Pilot study.

August 1999 Pilot DNR study – results reported here from two different amphipod toxicity tests (*Leptocheirus,* and *Ampelisca*).

Primarily focused on data from the National Coastal Assessment (NCA) Surveys in 2000 and 2001 at 54 stations

**Management Objective:** none

***Draft* Toxicity Indicator:** Statistical difference from control sample (percent survival compared to control)

**Data Analyses**

Data analyses primarily focused on the NCA 2000 and 2001 data. The amphipod, *Ampelisca*, was used to test for toxicity (5 reps). Reference sediment for the bioassay was collected from the Intercoastal Waterway, near the Florida-Alabama line. This sediment is a silty mud, relatively clean of chemical contaminants.

The results presented herein focus first on an unpublished pilot study conducted by DNR in the coastal bays during August 1999 (and the associated preliminary study to the pilot project conducted in July 1999) and second on the recent status analyses using 2000 and 2001 National Coastal Assessment survey results.

**A. 1999 Pilot DNR study:**

Pilot Study Methods: Each amphipod (*Ampelisca abdita* and *Leptocheirus plumulosus*) was subjected to a 10-day survival and growth bioassay. End-points employed were survival and growth. *Leptocheirus* was subjected to an additional 28-day test for survival, growth and reproduction. The bioassay tests were done at 25$^{o}$C in the temperature controlled Aquatic Toxicity Testing Laboratory at the University of Maryland Chesapeake Biological Laboratory at Solomons, Maryland in July and August of 1999.

Grain size analysis and chemical analyses for organic contaminants and mercury were also conducted on these sediments.

Both amphipods tests were performed in 25 ‰ salinity. They differed in their food sources: *Leptocheirus* was fed every three days with ground Tetramin™ while *Ampelisca* was fed with a mixture of diatoms Tahiti *Isochrysis* and *Skeletonema* daily.

Station 1 was located on the upstream side of the St. Martin River; Station 2 was at the mouth of the same River. Station 3 was where Turville and Herring creeks drain; Station 4 is located at Newport Bay near Sinepuxent Neck; and, Station 5 is located downstream from the Public Landing.

Control sediment was the sediment used as culture sediment for *Leptocheirus plumulosus*. The sediment was collected from a clean site in Fishing Bay at the mouth of the Transquaking River. The reference sand was collected at the mouth of the Patuxent River. *L. plumulosus* had been known to do better in sediment with 25% sand.

Results of Preliminary Bioassays (July 1999): Survival of the marine amphipod, *Ampelisca abdita*, was low during the preliminary bioassay test in July 1999. The control sediment only had 30% survival, which probably makes this test invalid. Station 1 had the lowest at 15%, while Station 2 ha the highest at 65%. Stations 3, 4 and 5 had 40% survival (Figure 5.3.2). The low survival could be attributed to stress during shipment and/or shortness of the salinity acclimation time (from 32‰ down to 25‰).

Growth of surviving *Ampelisca abdita* was between 49 - 96% over the bioassay period. Over the same period, amphipods in the control sediment increased 57% from the initial size. Station 3 had the highest growth at 96% while Station 5 had the lowest at 49% (Figure 5.3.2).

The number of animals used during the test (10 animals per replicate) was not ideal for this test, and we recommend at least 20 per replicate in future tests. *Ampelisca abdita* was recommended to be the test organism of choice for the Coastal Bays 2000 project.

Results of 1999 Pilot Study (August): *Ampelisca abdita* survival varied between 52 and 75%. The survival of the animals in the Control sediment was 72.5%, still a little lower than ideal survival (at, say, 75-80%). Stations 1 and 4 had the lowest survival at 52.5%

and Station 2 had the highest at 75% (figure 5.3.4). Survival, however, was low for the test to be valid. The surviving *Ampelisca abdita* increased a lot in size. Test animals the Control sediment increased 110%. Station 3 had the highest increase at 366% while the Station 4 had the lowest at 130%. The increase in sizes, however, did not vary significantly from those in the Control (Figure 3).

*Ampelisca* survival was somewhat low: Control only had 75% survival. Station 1 had the lowest at 63% and Station 3 had the highest at 80%. There was no significant difference in amphipod survival between the *L. plumulosus* in the Control sediment and those in the 5 sampling stations in the Coastal Bays. Low survival may have been the effect of the short salinity acclimation (from 15‰ to 25‰) time. Acclimatization should be at least a couple of months prior to bioassay tests.

The growth of *Ampelisca* varied between 184 and 368%. The size of the animals in the control sediment increased 278% in size from their initial weight. Station 1 had the highest increase at 368% while Station 4 had the lowest at 184%. This test, however, was only a 10-day test instead of the usual 28 days.

Survival of *Leptocheirus plumulosus* varied between 100 and 85 % after the 28-day exposure to the Control and the coastal bays sediments. The survival of the animals in the Control sediment was 96%. Station 3 had the highest survival at 100% while Station 5 had the lowest at 85%. There was no significant difference between survival of the Control sediment and the different Coastal Bays stations.

Percentage increase in size of *Leptocheirus* from most of the test stations was high compared to the control. *L. plumulosus* exposed to the sediment from Station 5 had the lowest increase in size (at 720%) of all the test animals. The amphipods in the Control sediment had the highest increase at 2400%, followed by those in Station 1 at 1962% and Station 2 at 1145%. The amphipods exposed to sediments from stations 3 and 4 had similar percentage increase in size (figure 5.3.5). *Leptocheirus* exposed to the sediment from Station 1 grew similarly to those in the Control sediment. Those exposed to the sediments from stations 2, 3 and 4 were significantly smaller ($p = 0.05$) than those in the Control while the animals in the sediment from Station 5 were also smaller ($p = 0.01$) than those in the Control.

*Leptocheirus* reproduction results were somewhat a similar to the percentage increases in size. The amphipods in the Station 1 sediment had higher number of progeny than those in the Control sediment. The number of progeny gets gradually lower from Station 2 down to Station 5. The number of young amphipods exposed to sediments from stations 4 and 5 were significantly different than the Control. Could the suggestion that some test animals produce more young when there is more environmental impact (to protect their population) be true?

<u>Pilot Study Summary</u>: The results of the two tests in the summer of 1999, using *Ampelisca abdita* was not consistent, maybe due to the fact that the animals did not have enough time to acclimate to the lower salinity, 25‰ (as compared to their natural

environment of around 32‰), they were subjected to during the bioassay tests. Maybe the temperature in the laboratory, 25°C ±2°C was higher than its natural environment. *Ampelisca* may have lower temperature requirement compared to *Leptocheirus*. These two environmental factors have to be considered should there be another opportunity to use *Ampelisca* as test animals for bioassay tests again.

On the other hand the *Leptocheirus plumulosus* were cultured in house, but they were subjected to somewhat similar acclimation to *Ampelisca*, the salinities were adjusted higher from their culture salinity of 15‰ to 25‰, in the same amount of time that the other amphipods were subjected to. They also had to adjust to the testing laboratory temperature which was 25°C ±2°C, from their culture laboratory of about 20°±2°C, however, this temperature change may not have been as high as what *Ampelisca* had been subjected to.

The results of the preliminary and the main (28-day) *Leptocheirus* tests were similar in the percentage survival of the test animals. The percentage increase in size during the 10-day test did not have a similar trend compared to the 28-day test. The results of both the growth (measured in terms of percentage increase in size) and reproduction (measured in terms of the number of juveniles) during the 28-day *Leptocheirus* test showed the same trend. Both were highest at Station 1, even higher than the Control in the number of juveniles; then both measures had a decreasing trend from stations 2 to 5.

Grain size, in particular clay content, has been shown to be related to sediment quality (see chapter on Total Organic Carbon by Wells). Results of grain size analysis for the sampling sites are given in Table 5.3.1.

Table 5.3.1: Results of grain size analyses done by the Maryland Geological Survey Team. (July samples were from the DNR preliminary study and August samples were from the DNR Pilot Study).

| | **Stations** | **% $H_2O$** | **Bulk Density** | **% Gravel** | **% Sand** | **% Silt** | **% Clay** | **Shepard's Classification** |
|---|---|---|---|---|---|---|---|---|
| July 9, 1999 samples | Control | 68.09 | 1.25 | 0.00 | 16.33 | 50.97 | 32.70 | Clayey Silt |
| | Station 1 | 73.87 | 1.20 | 0.00 | 2.20 | 34.53 | 63.27 | Silty Clay |
| | Station 2 | 57.61 | 1.37 | 0.00 | 2.12 | 59.01 | 38.87 | Clayey Silt |
| | Station 3 | 56.61 | 1.38 | 0.00 | 3.56 | 42.84 | 32.46 | Sand-Silt-Clay |
| | Station 4 | 57.36 | 1.37 | 0.00 | 24.70 | 70.41 | 26.03 | Clayey Silt |
| | Station 5 | 51.05 | 1.45 | 0.00 | 51.60 | 26.96 | 21.44 | Sand-Silt-Clay |
| August 1999 samples | Control | 72.63 | 1.21 | 0.00 | 8.38 | 54.95 | 36.68 | Clayey Silt |
| | Sand Ref* | 18.77 | 2.06 | 5.94 | 92.72 | 1.34 | 0.00 | Sand |
| | Station 1 | 65.31 | 1.28 | 0.00 | 4.41 | 36.82 | 58.77 | Silty Clay |
| | Station 2 | 52.58 | 1.43 | 0.00 | 2.68 | 60.83 | 36.49 | Clayey Silt |
| | Station 3 | 55.02 | 1.40 | 0.00 | 23.88 | 47.39 | 28.73 | Sand-Silt-Clay |
| | Station 4 | 51.16 | 1.45 | 0.00 | 10.34 | 68.36 | 21.30 | Clayey Silt |
| | Station 5 | 57.37 | 1.37 | 0.00 | 14.01 | 43.17 | 43.82 | Clayey Silt |

*Patuxent River sand.

Chemical Analyses conducted by the State Chemist Laboratory at the Maryland Department of Agriculture revealed fluoranthene, phenanthrene, benzo(a)pyrene, benz(e)acephenanthrylene an 1,2:5,6-dibenzanthracene were detected in sediments in most of the 5 stations, but not in the control (Fishing Bay) sediment (Figure 5.3.6). Mercury was found in sediments from all stations, including the control (Fishing Bay) sediment (Figure 5.3.7).

The presence of organic contaminants and mercury in the sediments showed somewhat similar trend to the *Leptocheirus plumulosus* test, especially the tests for percentage increase in size and the number of progeny. However, there were not enough samples and tests to make any conclusions.

## <u>B. Status of Sediment Toxicity (August 2000 and 2001)</u>

The following results refer to 10 day *Ampelisca* tests conducted as part of the National Coastal Assessment survey (bioassays done by Federal subcontractor).

*Assawoman Bay* – no toxicity detected at the 7 sites sampled (Figure 5.3.8).

*St. Martin River* – no toxicity detected at the 10 sites sampled (Figure 5.3.8).

*Isle of Wight*

In 2000, one site in the open bay showed evidence of toxicity; however, no toxicity was detected at same site in 2001 (Figure 5.3.8). Companion sediment chemistry data did not provide insight into what caused these results. Remaining six sites passed toxicity test.

*Sinepuxent* – No toxicity was detected at the 5 sites sampled in 2000 and 2001 (Figure 5.3.8).

*Newport* – No toxicity was detected at the 7 sites sampled in 2000 and 2001 (Figure 5.3.8).

*Chincoteague*

In 2000, one site at the north end of Chincoteague Island in Virginia (Wildcat Point) showed evidence of toxicity; however, no toxicity was detected at the same site in 2001 (Figure 5.3.8). Companion sediment chemistry data did not provide insight into what caused these results. Remaining 15 sites passed toxicity test in both years.

**Summary**

Overall, the Coastal Bays sediments show little evidence of toxicity. This is consistent with the sediment chemistry results that there were no exceedances of ER-M values (see previous chapter on Sediment Chemistry). No explanation for why the two sites that failed in 2000 but passed in 2001. It is important to note that dead-end canals were not sampled; other studies have shown these areas to have more toxicity due to leachate from pilings and runoff from development (Challiou et. al. 1996).

A pilot study conducted by DNR in 1999 showed that the amphipod test used for this status analysis is not as sensitive as other species. Recommend future testing for toxicity using other methods or trying to use more *Ampelisca,* say at least 20 per replicate, to see whether the animal is really a good indicator of an environmental impact. *Leptocheirus,* however, shows a lot of promise, even if it is not a strictly marine animal. It appears to be more sensitive to low levels of chemical contaminants. A longer acclimation time of approximately a month for *Ampelisca* (from 32 to 25 ‰) and a couple of months for *Leptocheirus* (from 15‰ to 25‰ might also prove beneficial. Recommend testing other 'pristine' sediment for future Control Sediment should be made. Maryland Geologic Survey suggested a couple of places in the Choptank River to collect from according to their metal content monitoring of different areas of the bay.

**Acknowledgements**

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**References**

Challiou, J.C., Weisberg, F.W., Kutz, F.W., DeMoss, T.E., Mangiaracina, L., Magnien, R., Eskin, R., Maxted, J., Price, K., and Summers, J.K., 1996, Assessment of the ecological condition of the Delaware and Maryland Coastal Bays, U.S. Environmental Protection Agency, National Human and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL., EPA/620/R-96/004.

USEPA 2002. Mid-Atlantic Integrated Assessment 1997-98 Summary Report, EPA/620/R-02/003. U.S. Environmental Protection Agency, Atlantic Ecology Division, Narragansett, RI.

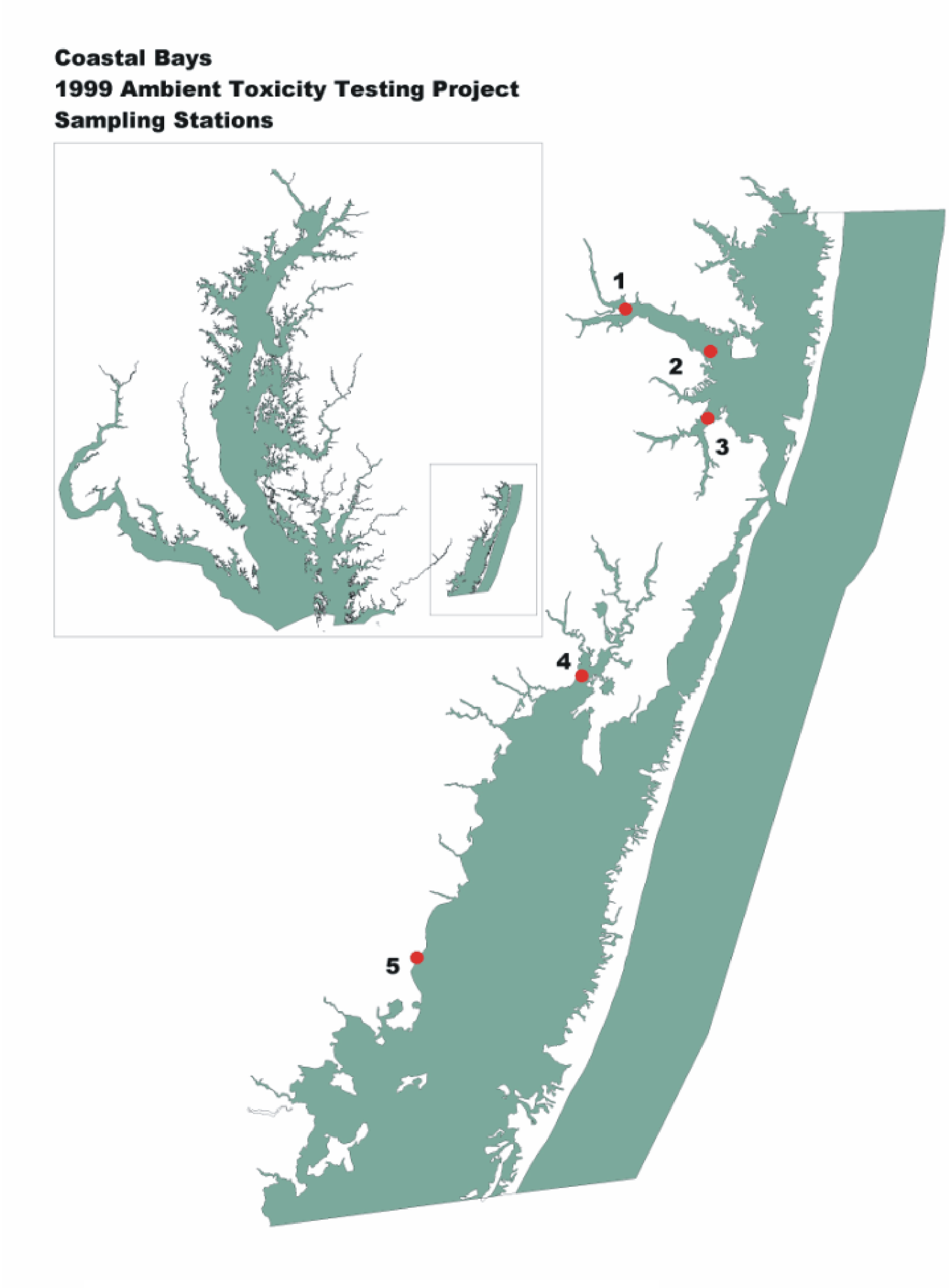


Figure 5.3.1: Map showing sites of sediment collection for 1999 pilot ambient toxicity testing.

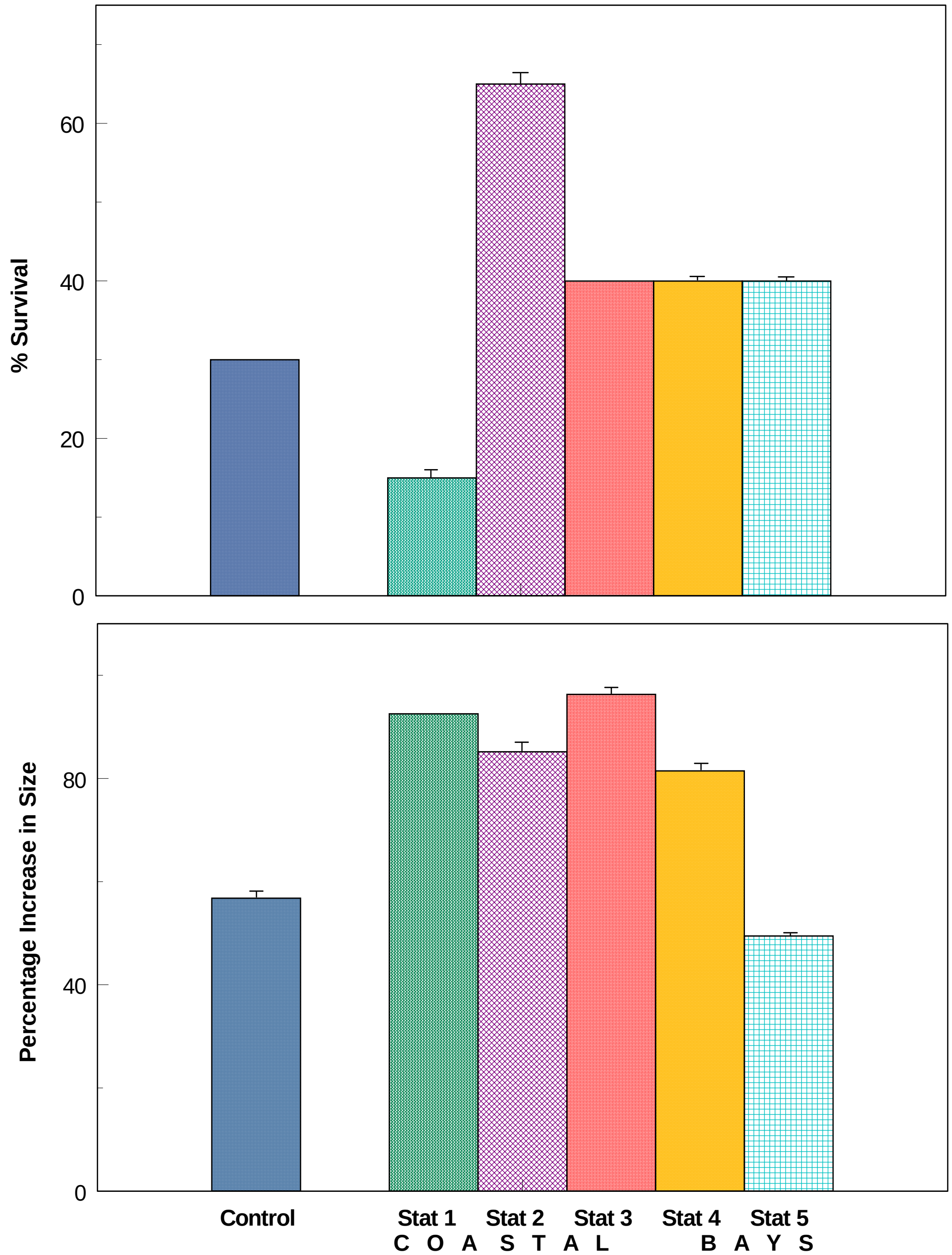


Figure 5.3.2: Preliminary Study (July 1999) bioassay using *Ampelisca abdita* percent survival and growth (percentage increase in size) after ten-day exposure to Coastal Bays sediments in July 1999. Control sediment was from Fishing Bay.

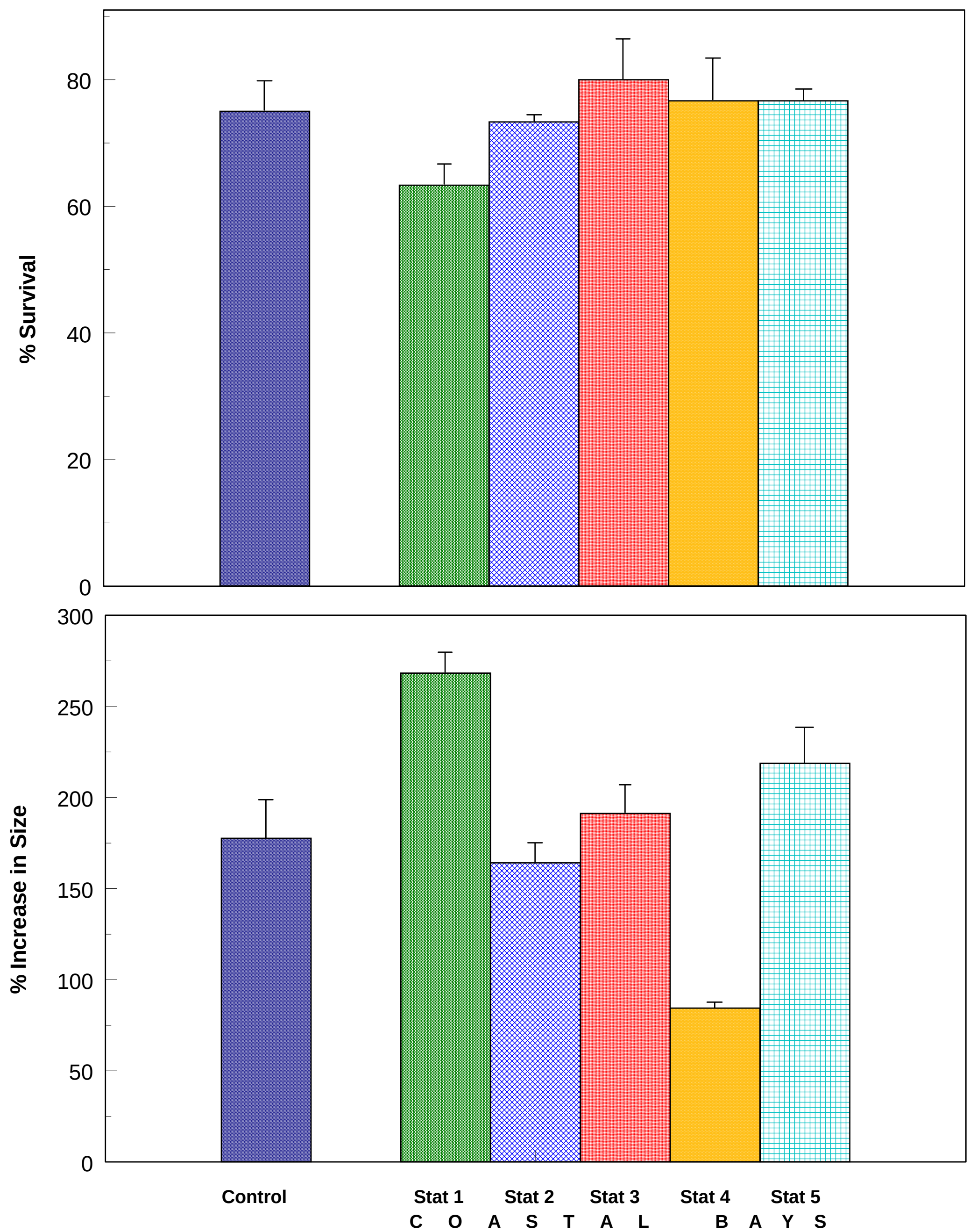


Figure 5.3.3: Preliminary DNR Study (July 1999) bioassay study using *Leptocheirus plumulosus* % survival and growth (% increase in size) after ten-day exposure to Coastal Bays sediments in 1999. Control sediment was from Fishing Bay.

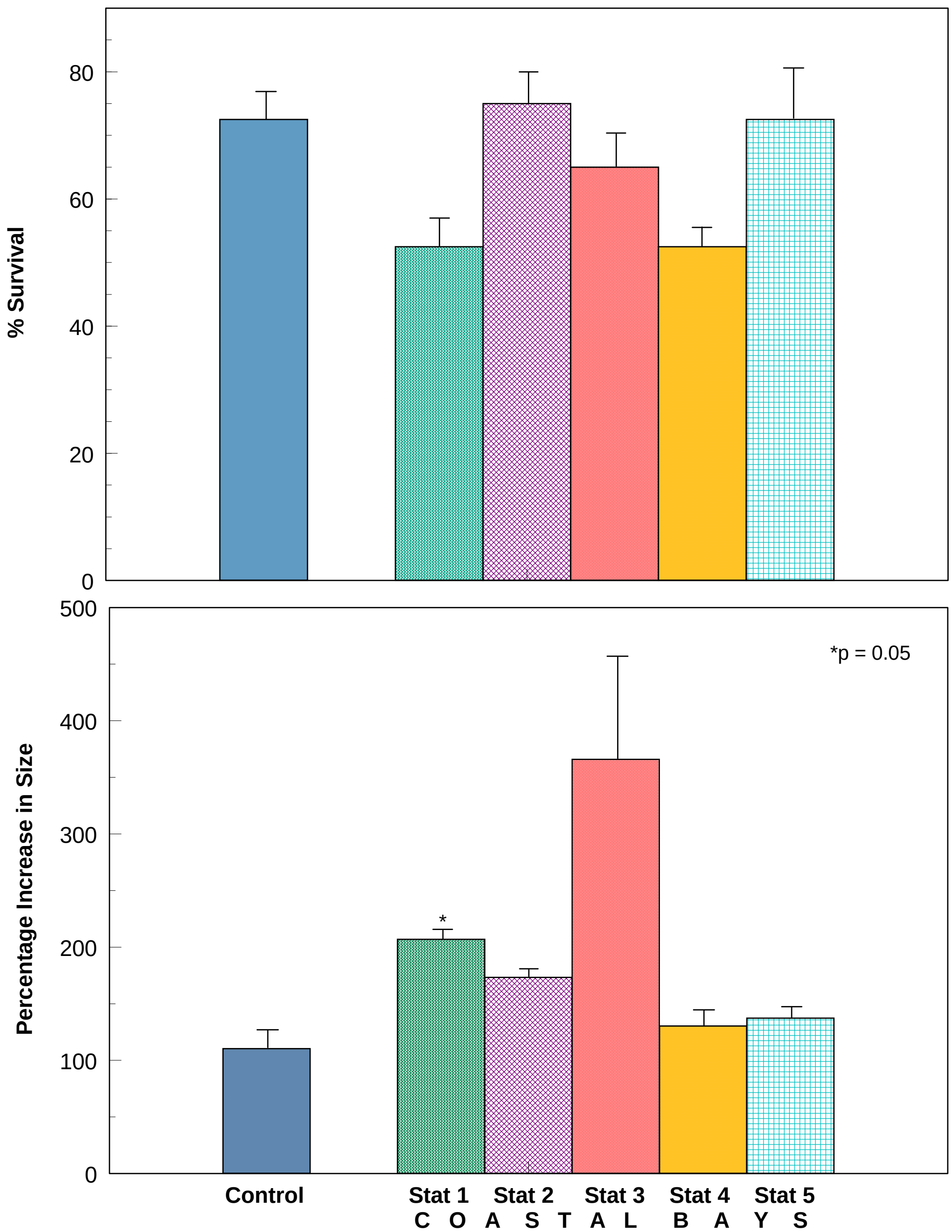


Figure 5.3.4: *Ampelisca abdita* % survival and growth (percentage increase in size) after ten-day exposure to Coastal Bays sediments in August 1999 (DNR Pilot Study). Control sediment was from Fishing Bay.

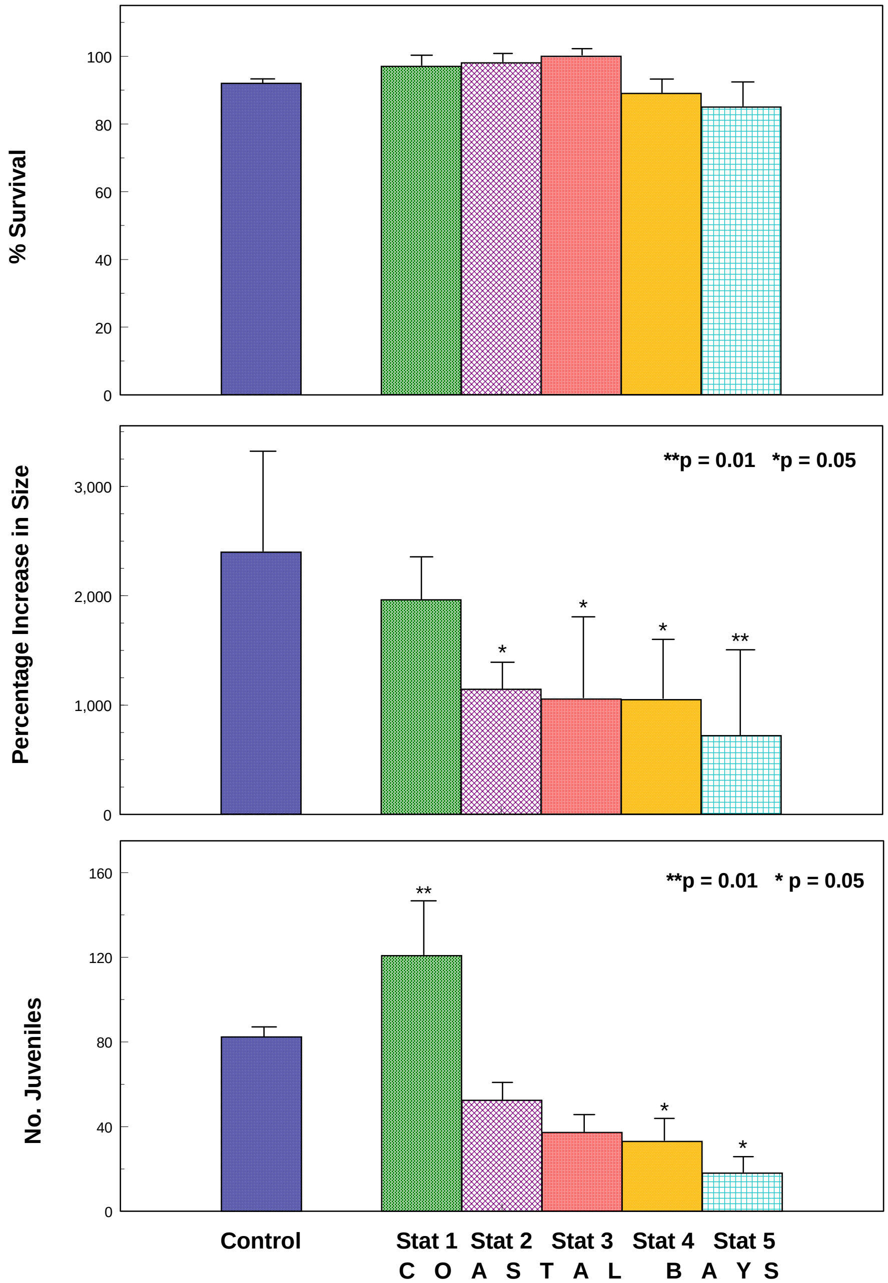


Figure 5.3.5: *Leptocheirus plumulosus* % survival, percentage increase in size and reproduction after 28-day exposure to Coastal Bays sediments in August1999 (DNR Pilot Study. Control sediment was from Fishing Bay.

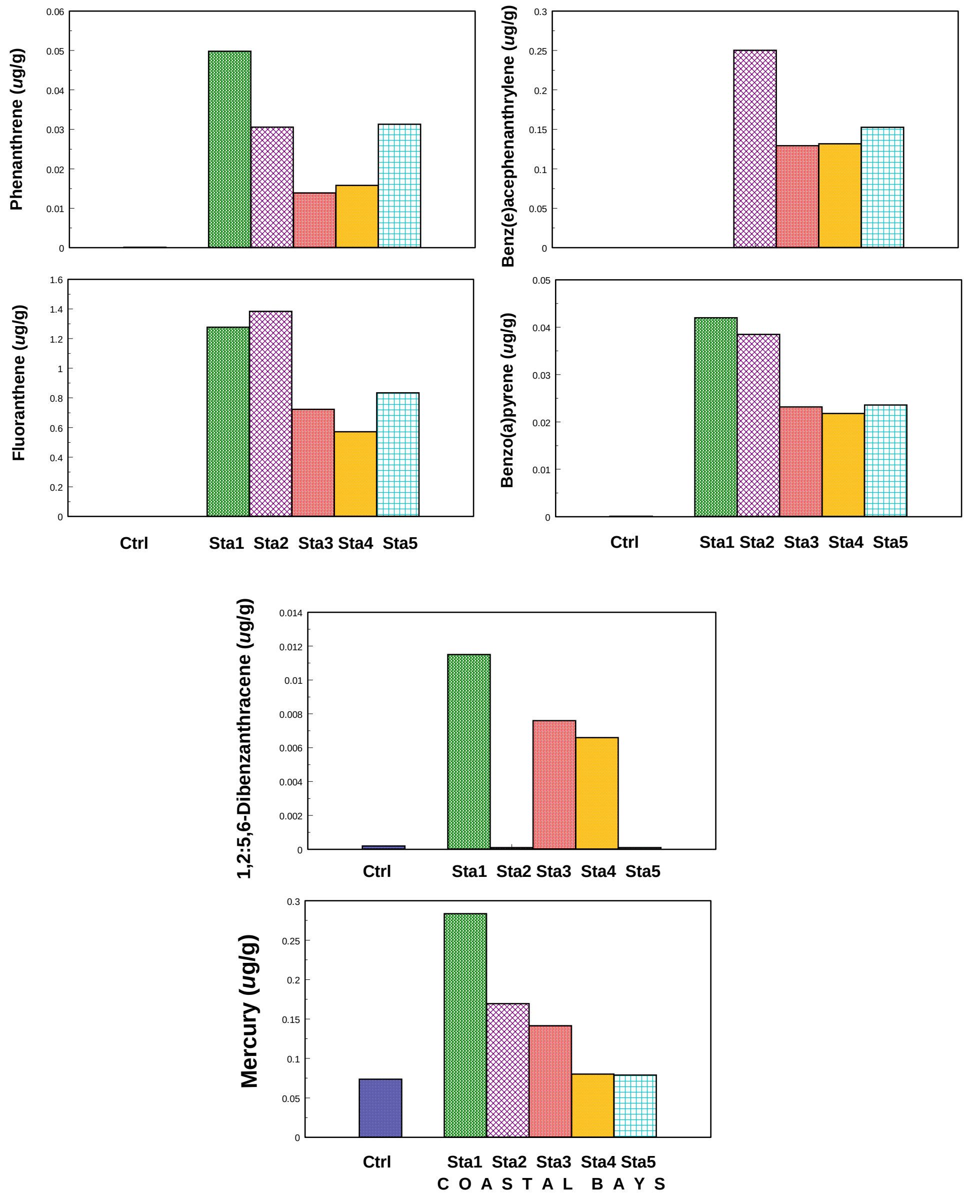


Figure 5.3.6: A comparison of selected organic contaminant and mercury concentrations between Coastal Bays and control (Fishing Bay) sediments. All concentrations are in micrograms per gram sediment (*u*g/g). a.) Phenanthrene b.) Benz(*e*)ancephenanthrylene c.) Fluoranthene d.) Benzo(*a*)pyrene e.) 1,2:5,6-Dibenzanthracene f.) Mercury.

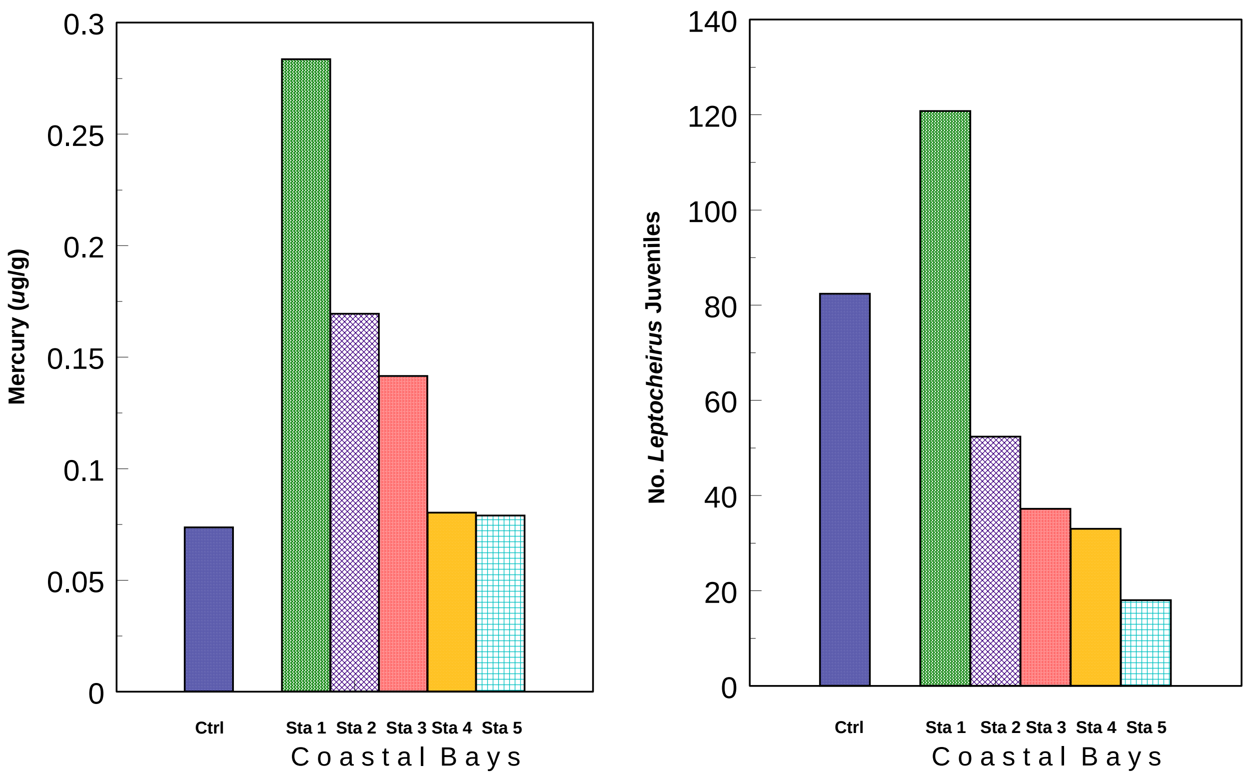


Figure 5.3.7: a.) Sediment mercury concentration from each Coastal Bays station and the control (Fishing Bay). b.) Number of juvenile *Leptocheirus plumulosus* found in sediments from each Coastal Bays station and the control (Fishing Bay).

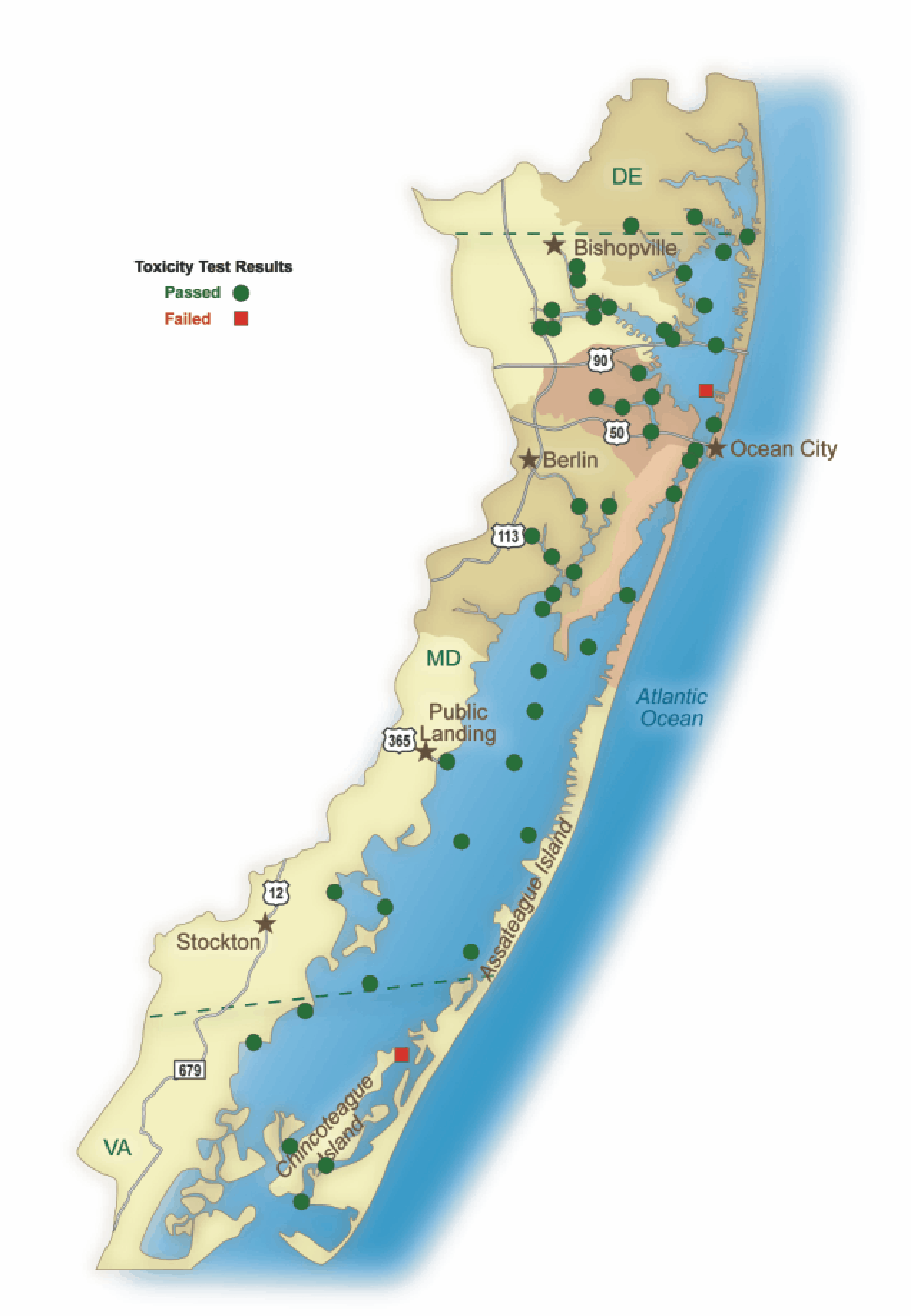


Figure 5.3.8: Map showing results of ambient toxicity tests conducted on samples collected in 2000 and 2001 (if failed either year).